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Characterization of Two Phospholipases A_2 in Serum of Patients with Sepsis and Acute Pancreatitis

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Summary: Pancreatic phospholipase A_2 and non-pancreatic ascitic phospholipases A_2 were studied in sera of healthy individuals and of patients suffering from sepsis or acute pancreatitis. In gel filtration experiments, immunoreactive ascitic phospholipase A_2, as determined in serum by a time-resolved fluoroimmunoassay, eluted either unassociated with an apparent M_r of 10 000 – 14 000 or associated with proteins of high molecular mass. Catalytically active ascitic phospholipase A_2 was associated with high molecular weight proteins. In acute pancreatitis the catalytically active and immunoreactive pancreatic phospholipase A_2 eluted mainly as a protein of M_r of 14 000. The results of the gel filtration experiments indicate that pancreatic phospholipase A_2 is not associated with other proteins in human serum, whereas ascitic phospholipase A_2 is associated with protein(s) of relative high molecular weight, or exists in different polymeric forms. We also purified phospholipase A_2 from sera of healthy individuals by ion exchange chromatography and HPLC. The enzyme was homogenous, displayed an M_r of approximately 13 500 as judged by SDS-polyacrylamide gel electrophoresis, and reacted with an antibody raised against ascitic phospholipase A_2.

Introduction

High concentrations of phospholipase A_2[1]) catalytic activity have been found in sera of patients suffering from a number of common and severe diseases, including septic shock (1), rheumatoid arthritis (2), acute pancreatitis (3), peritonitis (4) and multiple injuries (5). Secretory phospholipases A_2 have been classified into two groups on the basis of differences in their patterns of disulfide bonds. Group I phospholipases A_2 contain seven disulfide bridges. They are abundant in mammalian pancreatic juices and in cobra and sea snake venoms. Group II phospholipases A_2 are non-pancreatic in origin and lack the disulfide bridge between Cys 11 and 77 (6, 7).

The gene of a phospholipase A_2 belonging to group II has been cloned and its mRNA is present in numerous cells and tissues including human tonsils, kidney, rheumatoid synovial cells, spleen and lung (8, 9). The cytokines, interleukin-1 and tumour necrosis factor, increase the mRNA levels of group II phospholipase A_2 and the secretion of the enzyme (10, 11).

The mechanism of the elevation of extracellular group II phospholipase A_2 activity in inflammatory diseases remains unclear. Activation may result from the modification of a modulator protein or membrane structure (12). The catalytic activity of phospholipase A_2 in sera of patients with acute pancreatitis is not always pancreatic in origin. At least two immunologically distinct phospholipases A_2 have been detected (13).

We recently purified a phospholipase A_2 from the ascitic fluid of patients suffering from ovarian carcinoma and peritoneal carcinosis, and developed an immunoassay for its determination in body fluids, including serum and plasma (14). The cellular source of this enzyme is unknown. An antibody raised earlier

[1]) Enzymes:
Phospholipase A_2, phosphatide 2-acylhydrolase (EC 3.1.1.4)

against purified human pancreatic phospholipase A_2 (15) does not react with the ascitic phospholipase A_2 (14).

The purpose of the present work was to study the ascitic and pancreatic-type phospholipases A_2 in normal sera and in sera of patients suffering from acute pancreatitis or sepsis. We found that there are two pools of ascitic phospholipase A_2 in serum: free and protein-associated enzymes. In addition, we report here the isolation of phospholipase A_2 from human serum as a nearly homogeneous protein as judged by SDS-polyacrylamide gel electrophoresis.

Materials and Methods

Patients

Normal sera were obtained from three healthy laboratory workers. The sepsis sera were from hospitalized patients from the University Hospital of Ulm (Germany). Sera of a patients suffering from acute pancreatitis were from the University Central Hospital of Turku (Finland). Sera were stored at −20 °C until used.

Chromatography

Gel filtration chromatography of serum was performed on a 1.6 × 40 cm Sephadex G75 (Pharmacia, Uppsala, Sweden) column equilibrated with 20 mmol/l Tris-HCl (pH 8) buffer containing 0.15 mol/l NaCl. The column was eluted at a flow rate of 24 ml/h. The volumes of the chromatographed sera were 1–2 ml.

Phospholipase A_2 was purified from serum by ion exchange chromatography on a 1.6 × 10 cm CM-Sepharose CL6B (Pharmacia, Uppsala, Sweden) column equilibrated and washed free of anionic proteins with 2.5 mmol/l Tris-HCl (pH 7.5) containing 0.15 mol/l NaCl. The column was eluted stepwise with buffered 0.5 mol/l and 1.5 mol/l NaCl.

The phospholipase A_2-rich pool from ion exchange or gel filtration chromatography was applied to a reverse-phase C8-column (Aquapore Octyl RP 300, 0.46 × 22 cm, Brownlee Lab., Santa Clara, CA, USA) for HPLC. The column was equilibrated, and after application of the sample, washed free of unbound proteins with 1 ml/l trifluoroacetic acid. Phospholipase A_2 was eluted by using an acetonitrile gradient (95% acetonitrile/1 ml/l trifluoroacetic acid from 0 to 15% during 5 minutes and thereafter from 15 to 50% during 90 minutes.

Electrophoresis and immunoblotting

SDS-polyacrylamide gel electrophoresis was performed with the PhastSystem of Pharmacia (Uppsala, Sweden) using PhastGel gradient polyacrylamide gels (8–25%). Protein bands were visualized with Coomassie blue staining. Proteins were transferred to nitrocellulose filters with the PhastSystem semi-dry immunoblotting device as described by the manufacturer. Nitrocellulose filters were immunostained by using a Vectastain ABC-kit (Vector Laboratories Inc., Burlingame, CA, USA). Phospholipase A_2 purified from normal serum was detected with affinity-purified anti-ascitic phospholipase A_2 antiserum (7 mg/l). The phospholipase A_2 binding component was indentified using rabbit antiserum to human albumin (Dako, Glostrup, Denmark).

Phospholipase A_2 assays

The catalytic activity of phospholipase A_2 was measured radiochemically by using either *E. coli* (strain K12) labelled with [^{14}C]oleic acid (16) or a mixture of unlabelled dipalmitoylphosphatidylcholine (Sigma, St. Louis, MO, USA) and [2-^{3}H]dipalmitoyl-phosphatidylcholine (NEN, Boston, MA, USA) as substrate (17, 18). The enzyme proteins were measured immunochemically by time-resolved fluoroimmunoassays with antisera raised in rabbits against

1) the phospholipase A_2 purified from the ascitic fluid of patients with ovarian carcinoma and peritoneal carcinosis (ascitic fluid phospholipase A_2), and

2) the secretory pancreatic phospholipase A_2 purified from human pancreatic tissue (pancreatic phospholipase A_2), as described elsewhere (14, 19, for ascitic and pancreatic phospholipase A_2, respectively).

Protein determination

Protein was determined according to the method of *Lowry* et al. (20).

Statistical analysis

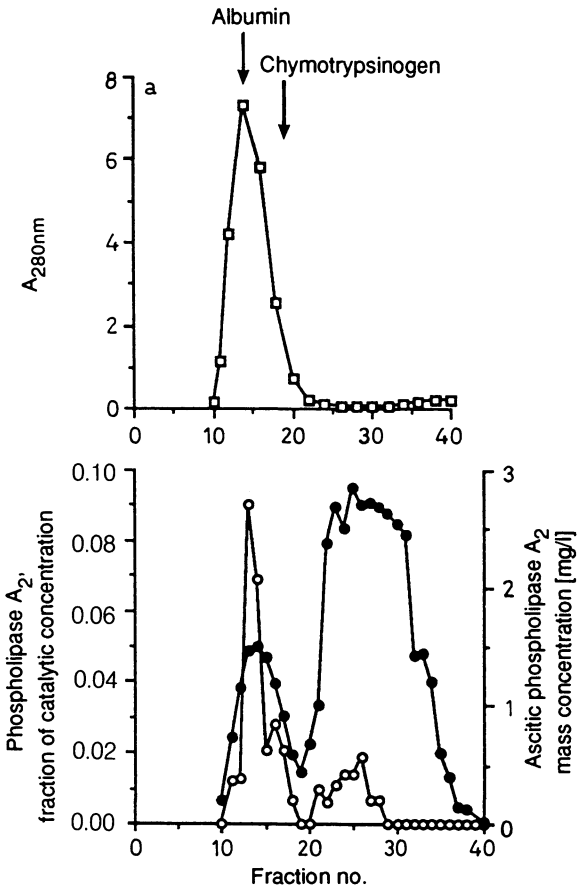
Student's t-test was used for the statistical analysis.

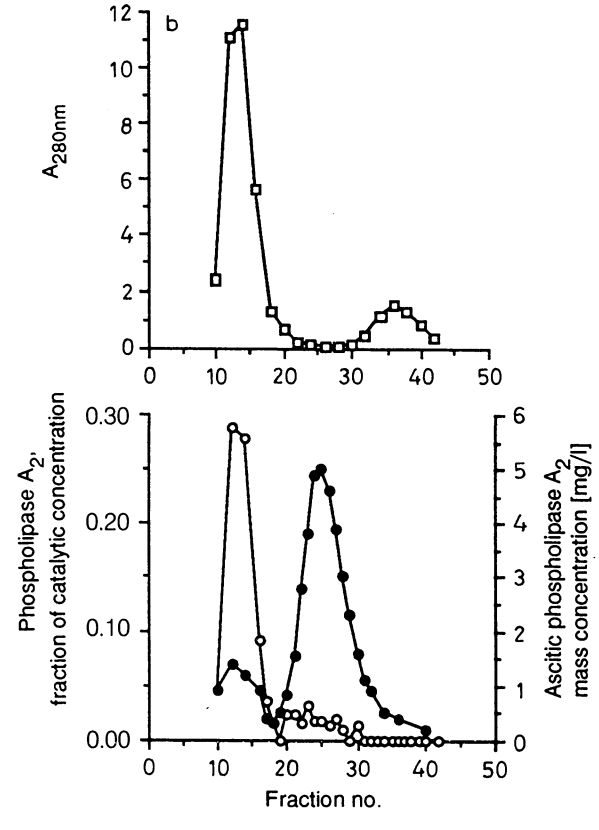
Results

Characterization of two phospholipases A_2 in serum in sepsis and acute pancreatitis

In gel filtration chromatography, immunoreactive ascitic phospholipase A_2 eluted in two peaks, one associated with high molecular mass proteins, and the other unassociated with an apparent M_r of 10 000–14 000 in normal serum and in sera of patients with sepsis and acute pancreatitis. Catalytically active phospholipase A_2 eluted mainly in association with high molecular-weight proteins in normal serum and in serum from a septic patients (fig. 1 a, b). In the serum of a patient with acute pancreatitis, the immunoreactivity and catalytic activity of pancreatic phospholipase A_2 eluted mainly as a protein of M_r about 14 000 (fig. 1 c). The catalytic activity and the content of pancreatic and ascitic phospholipase A_2 in the sera used in the gel filtration studies are presented in table 1. Table 2 gives the elution of the catalytic activity and immunoreactivity of pancreatic and ascitic phospholipase A_2 in the sera chromatographed on Sephadex G75.

The concentration of ascitic phospholipase A_2 was within the reference interval in patients suffering from sepsis ($p = 0.464$) or acute pancreatitis ($p = 0.6590$) (tab. 3). The concentration of pancreatic phospholipase A_2 was elevated in pancreatitis ($p = 0.0022$) but not in sepsis ($p = 0.1083$). The catalytic activity was clearly higher than normal in sepsis and pancreatitis ($p = 0.000$ and $p = 0.002$, respectively).





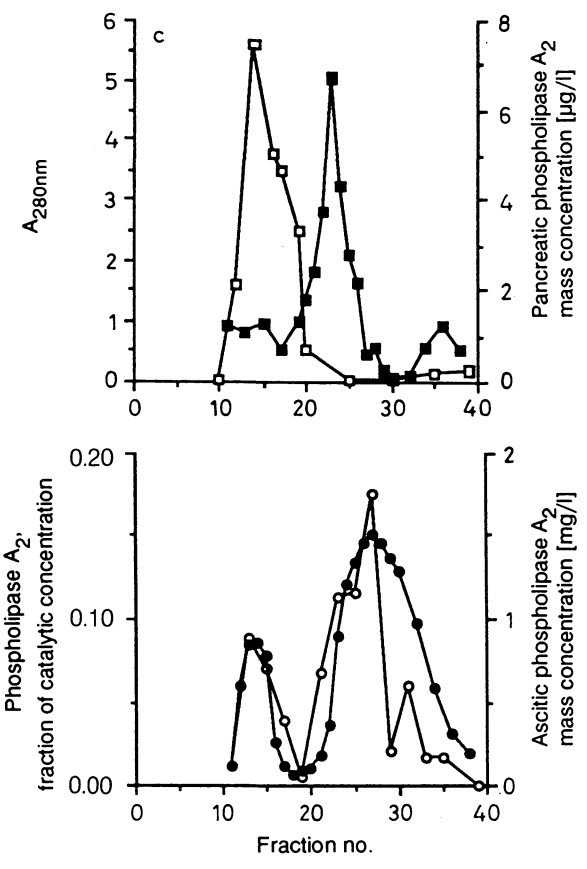


Fig. 1. Gel filtration chromatography of serum phospholipase A_2.Elution of normal serum (a), sepsis serum (b), and pancreatitis serum (c) from a Sephadex G75 column. Phospholipase A_2 immunoreactivity (●, ascitic phospholipase A_2; ■, pancreatic phospholipase A_2) was measured by time resolved fluoroimmunoassay and phospholipase A_2 catalytic activity (○) by using ^{14}C-labelled *E. coli* as substrate in the collected fractions as described under "Materials and Methods". Absorbance was measured at 280 nm (□). Elution of bovine serum albumin (M_r = 67 000) and chymotrypsinogen (M_r = 25 000) used as molecular mass markers is indicated by arrows.

Macromolecular phospholipase A_2 in serum

The fractions containing phospholipase A_2 associated with high molecular weight proteins after gel filtration chromatography were pooled and applied to a reverse phase column for HPLC. Phospholipase A_2 eluted at approximately 30 per cent of acetonitrile (14). After chromatography, phospholipase A_2-active fractions were incubated with NaSCN, and phospholipase A_2 was separated from the main protein peak by Sephadex G75 gel filtration chromatography (fig. 2). As a control experiment, phospholipase A_2-containing fractions obtained from reversed phase chromatography were chromatographed without NaSCN treatment on a Sephadex G75 column. Phospholipase A_2 eluted in the fraction of high molecular weight proteins (data not shown).

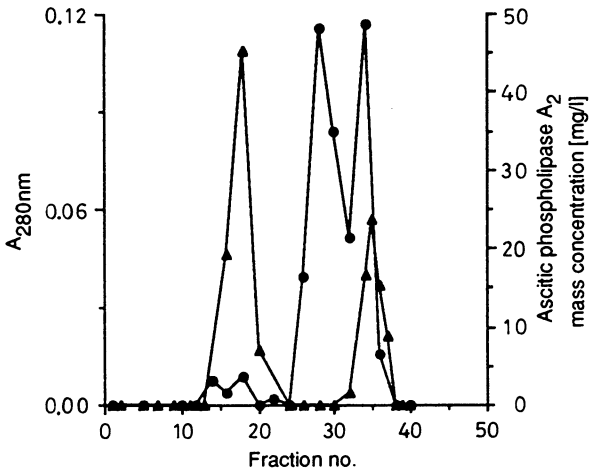


Fig. 2. Macromolecular phospholipase A_2 in serum. Phospholipase A_2-containing fractions from reverse phase chromatography on HPLC were pooled and incubated with 3.5 mol/l NaSCN in 50 mmol/l phosphate-buffered 0.15 mol/l NaCl (pH 7.3) for 10 minutes at room temperature. Phospholipase A_2 was separated from the main protein peak by Sephadex G75 as described in the section "Materials and Methods". Absorbance at 280 nm (▲) and immunoreactivity of ascitic phospholipase A_2 (●) were measured. The main protein peak eluted in fractions 15–20 and ascitic phospholipase A_2 in fractions 28–34.

Tab. 1. Sera used in gel filtration chromatography.

Serum	Ascitic phospholipase A_2 mg/l	Pancreatic phospholipase A_2 µg/l	Phospholipase A_2 U/l
Normal	107.2	5.0	2.2
Sepsis; male, 73a	104.5	5.1	12.7
Pancreatitis; male, 39a	138.4	61.4	5.2

SDS-polyacrylamide gel electrophoresis of the main peak (possibly a phospholipase A_2 binding protein) gave a single band of M_r approximately 67 000. Immunoblotting of this protein with the anti-albumin antibody gave a single band (fig. 3). The antibody against ascitic phospholipase A_2 did not recognize this protein as determined by time resolved fluoroimmunoassay (fig. 2).

Purification of phospholipase A_2 from normal serum

In ion exchange chromatography of normal serum, immunoreactive ascitic phospholipase A_2 was eluted in two discrete peaks and phospholipase A_2 catalytic activity in one peak by 1.5 mol/l NaCl (fig. 4). Only traces of immunoreactive ascitic phospholipase A_2 were eluted by 0.5 mol/l NaCl. The catalytically active phospholipase A_2 was found to represent only about 5 per cent of the total phospholipase A_2 in serum. The major fraction of phospholipase A_2 immunoreactivity (nearly 95 per cent of the total phospholipase A_2 in serum, fig. 4), which was contained in this peak, was further purified by HPLC. From the application of 20 ml of serum (1200 mg of protein) to the ion exchange column, the procedure yielded 0.490 mg of ascitic phospholipase A_2, devoid of catalytic activity. The apparent M_r of the purified serum-phospholipase A_2 was approximately 13 500 in SDS-polyacrylamide gel electrophoresis. The antibody against ascitic phospholipase A_2 recognized the purified phospholipase A_2 in immunoblotting (fig. 3).

Tab. 2. Summary of the results of gel filtration chromatography.

Serum	Relative molecular mass of phospholipase A_2	Ascitic phospholipase A_2 immunoreactivity	Pancreatic phospholipase A_2 immunoreactivity	Phospholipase A_2 catalytic activity
Normal	10 000–14 000	+	–	–
	67 000	+	–	+
Sepsis	10 000–14 000	+	–	–
	67 000	+	–	+
Pancreatitis	10 000–14 000	+	+	+
	67 000	+	–	+

Tab. 3. Catalytic activity concentration of phospholipase A_2 and mass concentration of ascitic phospholipase A_2 and pancreatic phospholipase A_2 in serum in acute pancreatitis (20 consecutive samples from seven patients) and sepsis (24 consecutive samples from four patients). Normal values were obtained from 20 healthy blood donors for catalytic activity of phospholipase A_2 and pancreatic phospholipase A_2 and from 20 healthy laboratory workers for ascitic phospholipase A_2. *Student*'s t-test was used for statistical analysis.

Patients	n	Catalytic concentration of phospholipase A_2		Ascitic phospholipase A_2		Pancreatic phospholipase A_2	
		(U/l)	p	(mg/l)	p	(µg/l)	p
Sepsis	24	13.7 (SD 8.7)	p = 0.0000	77.4 (SD 57.1)	p = 0.4604	10.4 (SD 13.8)	p = 0.1083
Pancreatitis	20	16.8 (SD 18.6)	p = 0.0023	72.3 (SD 37.8)	p = 0.6590	26.3 (SD 26.3)	p = 0.0022
Normal	20	2.1 (SD 0.8)		67.8 (SD 23.5)		5.5 (SD 1.2)	

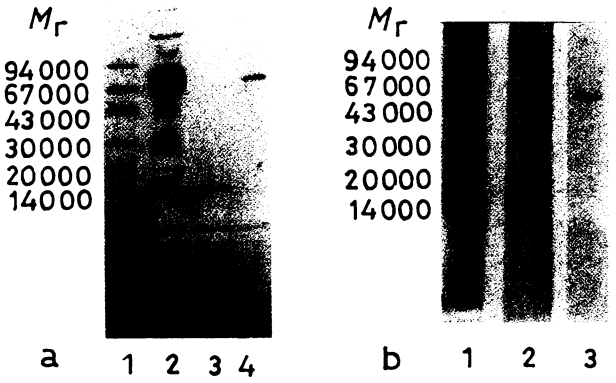


Fig. 3. SDS-polyacrylamide gel electrophoresis (a) and immunoblotting (b) of phospholipase A_2 and phospholipase A_2 binding component in serum.
a: Lane 1, molecular mass standards (M_r 14400–94000); lane 2, serum (1 : 40); lane 3, phospholipase A_2 purified from normal serum; lane 4, phospholipase A_2 binding component.
b: Detection of ascitic phospholipase A_2 and phospholipase A_2 binding component by immunoblotting as described in the section "Materials and Methods".
Lane 1, molecular mass standards stained with amidoblack (M_r = 14400–94000);
lane 2, phospholipase A_2 purified from normal serum;
lane 3, phospholipase A_2 binding component. Phospholipase A_2 was detected with the polyclonal rabbit antiascitic phospholipase A_2 antibody and phospholipase A_2 binding component with polyclonal rabbit antialbumin antibody.

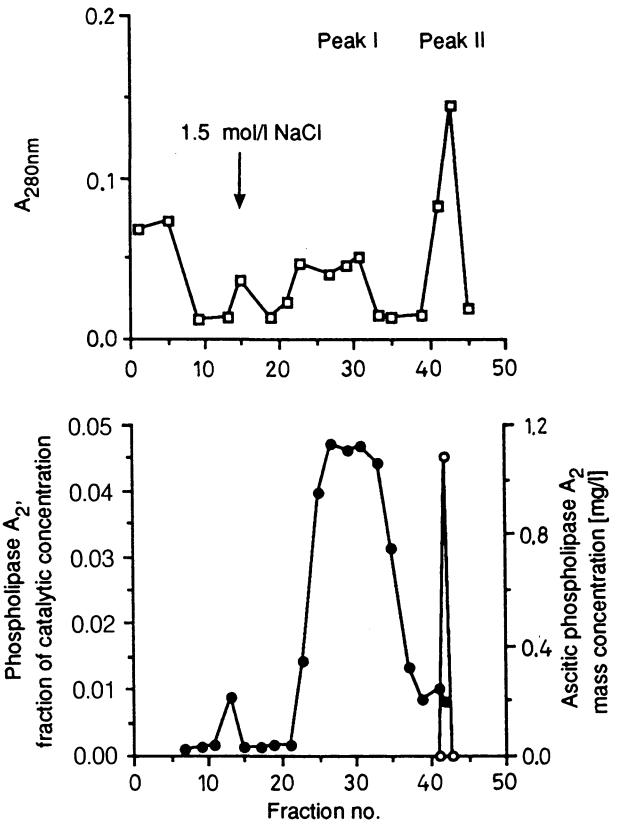


Fig. 4. Ion exchange chromatography of phospholipase A_2 in normal serum.
Serum was applied to a CM-Sepharose CL6B column. Phospholipase A_2 catalytic activity (○) was measured by using ^{14}C-labelled *E. coli* as substrate and immunoreactivity (●, phospholipase A_2) by time resolved fluoroimmunoassay as described under "Materials and Methods". Absorbance was measured at 280 nm (□). Fractions 25–33 (peak I) and 41–42 (peak II) were pooled. Each fraction contained 1.5 ml.

Discussion

Two phospholipases A_2 (ascitic and pancreatic phospholipase A_2) were characterized in normal serum and in sera of patients suffering from sepsis or acute pancreatitis. In gel filtration chromatography on Sephadex G75, ascitic phospholipase A_2 eluted with proteins of high (M_r 67000) and low (M_r 10000–14000) molecular mass as measured with time resolved fluoroimmunoassay. Pancreatic secretory phospholipase A_2 eluted as a protein of M_r about 14000. *Vadas* (1) reported that phospholipase A_2 from plasma in septic shock had an M_r of approximately 14000 in gel filtration chromatography on Sephadex G75. In the present study, we purified from normal serum a phospholipase A_2 that reacted with the antibody raised against ascitic fluid phospholipase A_2 but was devoid of catalytic activity. This enzyme protein had an M_r of approximately 13500. However, in our gel filtration experiments, the catalytic activity of phospholipase A_2 eluted mainly in association with high molecular weight protein(s), and only insignificant activity was found in the fractions containing proteins of low molecular mass.

The concentration of ascitic phospholipase A_2 in sera of patients suffering from acute pancreatitis were within the reference interval, but extremely high compared with the concentration of pancreatic phospholipase A_2 in the same serum samples. The possibility that the anti-ascitic-phospholipase A_2 antibody recognizes some non-phospholipase A_2 material cannot be excluded unequivocally by the methods used. However, we have excluded cross reactions with human albumin, human IgG, collagen type III and collagen type IV in the immunoassay for ascitic phospholipase A_2 (results not shown).

Cellular calcium-dependent phospholipases A_2 may be classified into at least two groups according to distinct characteristics of their primary structure (6). In addition to the pancreas, pancreatic-type phospholipase A_2 (group I) is present in cobra venom, spleen and lung (21–23). Phospholipases A_2 from platelet (24), spleen (25), liver (26) and ascitic fluid (27, 28)

resemble the phospholipases A_2 found in crotalic snake venoms in their primary structure and are classified in group II. Antibodies against group I phospholipases A_2 do not cross-react with group II phospholipases A_2 (29, 26, 30). Our results show that human ascitic phospholipase A_2 and pancreatic phospholipase A_2 are immunologically distinct proteins.

The present gel filtration studies indicate that ascitic phospholipase A_2 is partly associated with high molecular weight protein(s) in serum. Incubation of the phospholipase A_2-containing high molecular weight peak with NaSCN dissociates phospholipase A_2 from other proteins. Characterization of this protein by SDS-PAGE revealed one band of M_r approximately 67 000. Additionally, the anti-albumin antibody recognized this protein in immunoblotting. It was reported earlier that phospholipase A_2 binds to albumin (31). Binding to other proteins such as lipoproteins cannot be excluded.

Ascitic phospholipase A_2 may also exist in different polymeric forms in serum (32, 33). Rat lung contains, in addition to a high molecular weight phospholipase A_2, two minor fractions with phospholipase A_2 activity: a monomer (M_r 12 000) and a dimer (M_r 25 000) (34). An antiserum raised against *Naja naja* venom phospholipase A_2 cross-reacted with M_r 30 000 and 45 000 proteins from guinea pig alveolar macrophages and rat lymphocytes, and displayed a weak activity toward a M_r 14 000 protein (35). Similarly, an antibody against denatured rat platelet phospholipase A_2 detected three protein bands of M_r about 14 000, 32 000 and 44 000 in rat platelet lysate (36). In addition to low molecular mass phospholipases A_2 (37), enzymes of high molecular mass have been found in monocytic leukaemia cells (38, 39), rat serum (40), rat renal mesangial cells (41) and rat lung (34).

The results of the present gel filtration and immunoblotting experiments indicate that the ascitic phospholipase A_2 may either be associated with a protein of higher molecular weight, or exists in different polymeric forms in serum. Polymerization or protein association may change the catalytic properties of the enzyme.

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References

1. Vadas, P. (1984) Elevated phospholipase A_2 levels: Correlation with the hemodynamic and pulmonary changes in gram-negative septic shock. J. Lab. Clin. Med. *104*, 873–881.
2. Pruzanski, W., Vadas, P., Stefanski, E. & Urowitz, M. (1985) Phospholipase A_2 activity in sera and synovial fluids in rheumatoid arthritis and osteoarthritis. Its possible role as a proinflammatory enzyme. J. Rheumatol. *12*, 211–216.
3. Büchler, M., Malfertheiner, P., Shädlich, H., Nevalainen, T. J., Friess, H. & Beger, H. G. (1989) Role of phospholipase A_2 in human acute pancreatitis. Gastroenterology *97*, 1521–1526.
4. Büchler, M., Deller, A., Malfertheiner, P., Kleine, H. O., Wiedeck, H., Uhl, W., Samtner, M., Friess, H., Nevalainen, T. & Beger, H. G. (1989) Serum phospholipase A_2 in intensive care patients with peritonitis, multiple injuries and necrotizing pancreatitis. Klin. Wochenschr. *67*, 217–221.
5. Uhl, W., Büchler, M., Nevalainen, T. J., Deller, A. & Beger, H. G. (1990) Serum phospholipase A_2 in patients with multiple injuries. J. Trauma *30*, 1285–1290.
6. Heinrikson, R. L., Kreuger, E. T. & Keim, P. S. (1977) Amino acid sequence of phospholipase A_2-α from the venom of *Crotalus adamanteus*: A new classification of phospholipase A_2 based upon structural determinants. J. Biol. Chem. *252*, 4913–4921.
7. Fleer, E. A. M., Verheij, H. M. & de Haas, G. H. (1978) The primary structure of bovine pancreatic phospholipase A_2. Eur. J. Biochem. *82*, 261–269.
8. Ishizaki, J., Ohara, O., Nakamura, E., Tanaki, M., Ono, T., Kanda, A., Yoshida, N., Teraoka, H., Tojo, H. & Okamoto, M. (1989) cDNA cloning and sequence determination of rat membrane associated phospholipase A_2. Biochim. Biophys. Res. Commun. *162*, 1030–1036.
9. Kramer, R. H., Hession, C., Johansen, B., Hayes, G., McGray, P., Chow, E. P., Tizard, R. & Pepinski, R. B. (1989) Structure and properties of a human non-pancreatic phospholipase A_2. J. Biol. Chem. *264*, 5768–6775.
10. Nakano, T., Ohara, O., Teraoka, H. & Arita, H. (1990) Group II phospholipase A_2 mRNA synthesis is stimulated by two distinct mechanisms in rat vascular smooth muscle cells. FEBS Lett. *261*, 171–174.
11. Vadas, P. & Pruzanski, W. (1990) Phospholipase A_2 activation is the pivotal step in the effector pathway of inflammation. In: *The phospholipase A_2-role and function in inflammation* (Wong, P. Y-K. & Dennis, E. A., eds.) pp. 83–101, Plenum Press, New York.
12. Béréziat, G., Etienne, J., Kokkinidis, M., Olivier, J. L. & Pernas, P. (1990) New trends in mammalian non-pancreatic phospholipase A_2 research. J. Lipid. Mediat. *2*, 159–172.
13. Eskola, J. U., Nevalainen, T. J. & Kortesuo, P. (1988) Immunoreactive pancreatic phospholipase A_2 and catalytically active phospholipase A_2 in serum from patients with acute pancreatitis. Clin. Chem. *34*, 1052–1054.
14. Kortesuo, P. T. & Nevalainen, T. J. (1991) Phospholipase A_2 in human ascitic fluid: Purification characterization and immunochemical detection. Biochem. J. *278*, 263–267.
15. Eskola, J. U., Nevalainen, T. J. & Aho, H. J. (1983) Purification and an characterization of human pancreatic phospholipase A_2. Clin. Chem. *29*, 1772–1776.
16. Patriarca, P., Beckerdite, S. & Elsbach, P. (1972) Phospholipases and phospholipid turnover in *Escherichia coli* spheroplasts. Biochim. Biophys. Acta *260*, 593–600.
17. Schädlich, H., Büchler, M. & Berger, H. G. (1987) Improved method for the determination of phospholipase A_2 catalytic activity concentration in human serum and ascites. J. Clin. Chem. Clin. Biochem. *25*, 505–509.

18. Schädlich, H. R., Büchler, M. & Beger, H. G. (1989) A radiochemical test for phospholipase A_2 catalytic activity. Klin. Wochenschr. *67*, 110–112.
19. Eskola, J. U., Nevalainen, T. J. & Lövgren, T. N-E. (1983) Time-resolved fluoroimmunoassay of human pancreatic phospholipase A_2. Clin. Chem. *29*, 1777–1780.
20. Lowry, O., Rosenbrough, N., Farr, A. & Randall, R. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*, 265–275.
21. Tojo, H., Ono, T., Kuramitsu, S., Kagamiyama, H. & Okamoto, M. (1988) A phospholipase A_2 in the supernatant fraction of rat spleen – Its similarity to rat pancreatic phospholipase A_2. J. Biol. Chem. *263*, 5724–5731.
22. Sakata, T., Nakamura, E., Tsuruta, Y., Tamaki, M., Teraoka, H., Tojo, H., Ono, T. & Okamoto, M. (1989) Presence of pancreatic-type phospholipase A_2 mRNA in rat gastric mucosa and lung. Biochim. Biophys. Acta *1007*, 124–126.
23. Tatsumi, H., Tojo, H., Senda, T., Ono, T., Fujita, H. & Okamoto, M. (1990) Immunocytochemical studies on the localization of pancreatic-type phospholipase A_2 in rat stomach and pancreas, with special reference to the stomach cells. Histochemistry *94*, 135–140.
24. Horigome, K., Hayakawa, M., Inoue, K. & Nojima, S. (1987) Purification and characterization of phospholipase A_2 released from rat platelets. J. Biochem. *Tokyo) 101*, 625–631.
25. Ono, T., Tojo, H., Kuramitsu, S., Kagamiyama, H. & Okamoto, M. (1988) Purification and characterization of a membrane-associated phospholipase A_2 from rat spleen: its comparison with a cytosolic phospholipase A_2 S-1. J. Biol. Chem. *263*, 5732–5738.
26. Aarsman, A. J., deJong, J. G. N., Arnoldussen, E., Neys, F. W., van Wassenaar, P. D. & van den Bosch, H. (1989) Immunoaffinity purification, partial sequence, and subcellular localization of rat liver phospholipase A_2. J. Biol. Chem. *264*, 10008–10014.
27. Forst, S., Weiss, J. & Elsbach, P., Maraganore, J. M., Reardon, I. & Heinrikson, R. L. (1986) Structural and functional properties of a phospholipase A_2 purified from an inflammatory exudate. Biochemistry *25*, 8381–8285.
28. Wright, G. W., Ooi, C. E., Weiss, J. & Elsbach, P. (1990) Purification of a cellular (granulocyte) and an extracellular (serum) phospholipase A_2 that participate in the destruction of *Escherichia coli* in rabbit inflammatory exudate. J. Biol. Chem. *265*, 6675–6681.
29. deJong, J. G. N., Amesz, H., Aarsman, A. J., Lenting, H. B. M. & van den Bosch, H. (1987) Monoclonal antibodies against an intracellular phospholipase A_2 from rat liver and their cross-reactivity with other phospholipase A_2. Eur. J. Biochem. *164*, 129–135.
30. Inada, M., Tojo, H., Kawata, S., Tarui, S. & Okamoto, M. (1991) Preferential distribution of group-II-like phospholipase A_2 in mononuclear phagocytic cells in rat spleen and liver. Eur. J. Biochem. *197*, 323–329.
31. Elsbach, P. & Pettis, P. (1973) Phospholipase A_2 associated with serum albumin. Biochim. Biophys. Acta *296*, 89–93.
32. Cordella-Miele, E., Miele, L. & Mukherjee, A. B. (1990) A novel transglutaminase-mediated post-translational modification of phospholipase A_2 dramatically increases its catalytic activity. J. Biol. Chem. *265*, 17180–17188.
33. Schalkwijk, C. G., Märki, F., Wiesenberg, I. & van den Bosch, H. (1991) The detection of multimeric forms of phospholipase A_2 upon sodium dodecyl sulfate-polyacrylamide electrophoresis. J. Lipid Mediators *4*, 83–96.
34. Lindahl, M., von Schenk, H. & Tagesson, C. (1989) Isolation and characterization of phospholipase A_2 from rat lung with affinity chromatography and two-dimensional gel electrophoresis. Biochim. Biophys. Acta *1005*, 282–288.
35. Masliah, J., Kadiri, C., Pepin, D., Rybkine, T., Etienne, J., Chambaz, J. & Bérézíat, G. (1987) Antigenic relatedness between phospholipase A_2 from *Naja Naja* venom and from mammalian cells. FEBS Lett. *222*, 11–16.
36. Murakami, M., Kudo, I., Natori, Y. & Inoue, K. (1990) Immunochemical detection of platelet type phospholipase A_2 in the rat. Biochim. Biophys. Acta *1043*, 34–42.
37. Vadas, P. & Pruzanski, W. (1986) Biology of disease. Role of secretory phospholipase A_2 in the pathology of disease. Lab. Invest. *55*, 391–404.
38. Clark, J. D., Milona, N. & Knopf, J. L. (1990) Purification of a 110-kilodalton cytosolic phospholipase A_2 from the human monocytic cell line U937. Proc. Natl. Acad. Sci. U. S. A. *87*, 7708–7712.
39. Diez, E. & Mong, S. (1990) Purification of phospholipase A_2 from the human monocytic leukemic U937 cells. J. Biol. Chem. *265*, 14654–14661.
40. Aarsman, A. J., Roosenboom, C. F. P., van Geffen, G. E. W. & van den Bosch, H. (1985) Some aspects of rat platelet and serum phospholipase A_2 activities. Biochim. Biophys. Acta *837*, 288–295.
41. Gronich, J. H., Bonventre, J. V. & Nemenoff, R. A. (1988) Identification and characterization of a hormonally regulated form of phospholipase A_2 in rat renal mesangial cells. J. Biol. Chem. *263*, 16645–16651.

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